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Lacto-*N*-Fucopentaose III (Lewis^x), a Target of the Antibody Response in Mice Vaccinated with Irradiated Cercariae of *Schistosoma mansoni*

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Carbohydrates on soluble egg antigens are major epitopes for the antibody responses of patients and mice infected with *Schistosoma mansoni*. Recently, protective sera of mice vaccinated with irradiated cercariae were shown to recognize carbohydrate epitopes on schistosomal glutathione *S*-transferase. The present study demonstrates that carbohydrate epitopes are major targets of sera from C57BL/6J and CBA/J mice vaccinated with 15- or 50-kilorad-irradiated cercariae of *S. mansoni*. Antibody titers to carbohydrate epitopes increased with the number of vaccinations and were considerably higher in C57BL/6J mice than in CBA/J mice. The specificity of this anticarbohydrate response was determined by measuring antibody binding to defined oligosaccharide residues known to be present on the parasite. A predominant target of the humoral anticarbohydrate response of vaccinated mice was lacto-*N*-fucopentaose III, a molecule relevant for cell trafficking. We observed no binding to its nonfucosylated homolog, lacto-*N*-neotetraose, or to oligosaccharides present on keyhole limpet hemocyanin. The strongest antibody response to lacto-*N*-fucopentaose III was observed for C57BL/6J and CBA/J mice repeatedly vaccinated with 15-kilorad-irradiated cercariae, which also achieve the highest levels of protection. Immunoglobulin M was the predominant antibody class binding to lacto-*N*-fucopentaose III. We conclude that in the irradiated-cercaria vaccine model, C57BL/6J and CBA/J mice produce anticarbohydrate antibodies against various stages of *S. mansoni* and that the oligosaccharide lacto-*N*-fucopentaose III is one target of this response. Lacto-*N*-fucopentaose III and its specific antibodies may profoundly affect host resistance and parasite homing.

In experimental murine schistosomiasis, the highest levels of resistance to challenge infections have been achieved by vaccination with live, radiation-attenuated cercariae (3, 17, 18). Induction of this resistance requires functional humoral and cellular immune compartments (19). Sera obtained from mice vaccinated repeatedly with irradiated cercariae of *Schistosoma mansoni* (VMS) are capable of protecting naive mice against challenge infection (4, 12, 18). Previous studies have shown that VMS contain lower levels of antibodies recognizing surface membrane epitopes on schistosomula than sera from chronically infected mice (CMS) (15, 16). The difference was attributed to the observation that CMS contained antibodies binding cross-reactive carbohydrate (CHO) epitopes on schistosomula and on eggs and, further, that VMS lacked such antibodies. In contrast, we reported that approximately half of the antibodies in VMS that are specific for glutathione *S*-transferase (GST) bind to CHO epitopes on this antigen (18). This discrepancy led us to examine CHO-binding antibodies in VMS with a broad array of crude antigen preparations from different stages of the parasite.

The identification of specific CHO epitopes recognized by VMS is of interest, because certain oligosaccharides involved in cell interactions of the host's immune system are also present on the parasite's surface. One of these ubiquitous sugars is lacto-*N*-fucopentaose III (LNFP-III), also known as mammalian stage-specific embryonic antigen 1 (SSEA1) (5, 7). LNFP-III contains the trisaccharide Lewis^x which is a ligand for P-selectin CD62 (PADGEM and GMP140) (9, 10, 21). Recently, LNFP-III was demonstrated to be also involved in directing $CD4^+$ T-cell subset responses of the host (24). As a result, antibodies specific for these oligosaccharides may have profound effects on host-parasite interactions. Thus, we aimed to identify whether particular CHO epitopes are recognized by VMS. To evaluate the relevance of CHO-specific antibodies for protection in the vaccine model, we compared their titers in various VMS obtained from mice with different levels of resistance.

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MATERIALS AND METHODS

Abbreviations. C57 mice, C57BL/6J mice; CBA mice, CBA/J mice; 1-15, vaccinated once with 15-kilorad-irradiated cercariae; 1-50, vaccinated once with 50-kilorad-irradiated cercariae; 3-15, vaccinated three times with 15-kilorad-irradiated cercariae; 3-50, vaccinated three times with 50-kilorad-irradiated cercariae; 3B11, MAb recognizing stage-specific embryonic antigen 1; IgG3, immunoglobulin G3; CERC, saline-soluble preparation of cercarial antigens; DOC, deoxycholate; DOC-AD, DOC extracts of adult worm antigens; DOC-CERC, DOC extracts of cercarial antigens; DOC-SOM, DOC extracts of schistosomular antigens; ELISA, enzyme-linked immunosorbent assay; E3, anti-egg MAb recognizing keyhole limpet hemocyanin; E5, anti-egg MAb recognizing LNFP-III; HSA, human serum albumin; KLH, keyhole limpet hemocyanin; LNT, lacto-*N*-neotetraose; MAb, monoclonal antibody; NMS, normal mouse sera; OD, optical density; PBS, phosphate-buffered saline; PBS-T0.3, PBS containing 0.30% Tween 20; PBS-T0.05, PBS containing 0.05% Tween 20; SCHLAP, saline-soluble preparation of schistosomular antigens; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SEA, saline-soluble preparation of egg antigens; SWAP, saline-soluble preparation of adult worm antigens.

Host animals and parasites. Female C57 and CBA mice were purchased from Bomholtgard (Ry, Denmark) and Jackson Laboratory (Bar Harbor, Maine). A Puerto Rican strain of *S. mansoni* was maintained in our laboratory by passage through *Biomphalaria glabrata* snails and Swiss Webster mice (Taconic Farms, Germantown, N.Y.). Parasites of various stages were also obtained from F. Lewis, Biomedical Research Institute, Rockville, Md.

Exposure of mice to irradiated cercariae. At monthly intervals, groups of 6- to 8-week-old C57 and CBA mice were exposed to irradiated cercariae of *S. man-*

soni. Freshly harvested cercariae were diluted to 500 organisms per ml and immediately attenuated with an irradiation dose of either 15 or 50 kilorads emitted by a ^{60}Co source. Mice anesthetized by intramuscular injection with a mixture of ketamine-HCl (0.08 mg/g) (Aveco, Fort Dodge, Iowa) and xylazine (0.005 mg/g) (Haver, Shawnee, Kansas) were exposed to 500 irradiated cercariae for 30 min via their shaved abdomen by the ring method (20).

Sera. Vaccine sera were obtained by bleeding vaccinated C57 and CBA mice from the tail 26 days after each exposure to cercariae. CMS were obtained from C57 and CBA mice 20 to 24 weeks after an infection with 25 to 30 nonattenuated cercariae of *S. mansoni*. Sera from strain- and age-matched naive mice served as a negative control (NMS). For each experimental group, sera from at least 10 mice were pooled.

MAbs. Anti-egg MAb E3 (IgG3) and E5 (IgM) have been produced by immunization with living schistosome eggs or SEA (2). The nonprotective MAb E3 binds to an as yet unidentified oligosaccharide epitope on eggs that is also present on KLH. The protective MAb E5 recognizes the oligosaccharide LNFP-III that contains the Lewis^x trisaccharide. MAb 3B11, specific for SSEA1 (a gift from Ursula Dräger, Harvard Medical School, Boston, Mass.), is of the IgM class and recognizes LNFP-III in mammalian neural tissue and on schistosomes (5).

Parasite antigens. CERC, SCHLAP, SWAP, or SEA were produced from previously frozen cercariae, living mechanically transformed schistosomula (11), previously frozen adult worms, or from freshly isolated eggs (2), respectively. With a glass homogenizer, organisms were homogenized in PBS containing 1 μM leupeptin (Boehringer Mannheim, Indianapolis, Ind.) for 1 h on ice. After centrifugation at 16,000 × *g* for 1 h at 4°C, supernatants were collected, aliquoted, and stored at −80°C. To enrich for membrane proteins, DOC extracts were prepared from freshly harvested cercariae (DOC-CERC) or live, mechanically transformed schistosomula (DOC-SOM) by resuspending the washed organisms in a solution containing 4 mM DOC (Sigma, St. Louis, Mo.), 28 mM Tris base (Sigma), and 1 μM leupeptin, pH 8.3. Extraction was carried out for 1 h at 4°C on a nutator without mechanical disruption of the organisms. DOC extracts of frozen adult worms (DOC-AD) were prepared with a glass homogenizer as described above for saline-soluble preparations. After centrifugation, the supernatants were collected and stored as described above. Protein contents were estimated by the Micro-BCA colorimetric assay (Pierce, Rockford, Ill.).

Defined oligosaccharides and KLH. LNFP-III and its nonfucosylated homolog, LNT, were purchased conjugated to HSA at a ratio of 10 to 20 oligosaccharides for each HSA molecule (Accurate Chemical & Scientific Corp., Westbury, N.Y.). HSA was purchased from Sigma, and KLH was purchased from Boehringer Mannheim.

ELISA with and without sodium metaperiodate treatment of antigens. Antigens were diluted in PBS at the following concentrations: parasite antigens at 5 μg/ml, LNFP-III–HSA and LNT-HSA at 1 μg/ml, HSA at 0.89 μg/ml, and KLH at 10 μg/ml. Fifty microliters of antigen was dispensed into each well of an Immulon 2 ELISA plate (Dynatech, Chantilly, Va.) and incubated overnight at 4°C in a humidified chamber. Wells were blocked with PBS-T0.3 (Sigma). Plates in which antigens were to be treated with sodium metaperiodate (Sigma) were washed twice with 0.05 M sodium acetate buffer (pH 4.5) (26) and then incubated with sodium metaperiodate in 0.05 M sodium acetate buffer (pH 4.5) at concentrations of 0, 1, 10, and 20 mM for 1 h at room temperature in the dark. The wells were washed with sodium acetate buffer. Subsequently, aldehyde groups were blocked by treatment with 50 mM sodium borohydrate (Sigma) for 30 min at room temperature. For all ELISAs, plates were washed five times with PBS-T0.05. VMS, CMS, and NMS were dispensed into wells at a dilution of 1:800 or 1:1,600 in PBS-T0.3, and MAbs were used as undiluted culture supernatants or diluted to a concentration of 10 μg/ml in PBS-T0.3. Primary antibodies were incubated for 1 h at room temperature. The wells were washed five times with PBS-T0.05 and incubated with peroxidase-conjugated goat anti-mouse IgG + IgM, IgG, or IgM antibodies (Boehringer Mannheim) or biotin-labeled rabbit anti-mouse IgA (Pharmingen, San Diego, Calif.) for 1 h at room temperature. Wells incubated with anti-IgA antibody were washed and incubated with strepavidin (Sigma) at a dilution of 1:1,000 for 1 h at room temperature. After the wells were washed with PBS-T0.05, tetramethylbenzidine substrate (Kirkegaard & Perry, Gaithersburg, Md.) was added. The reaction was stopped with phosphoric acid at a concentration of 1:40, and the OD of each well was read at 450 nm in an automatic ELISA reader (UVmax; Molecular Devices, Menlo Park, Calif.). For each antigen, all sera were tested simultaneously on the same plate. Each assay was repeated two or three times. OD values shown are the means of duplicate wells of a representative experiment.

SDS-PAGE and Western blot (immunoblot) analysis. SDS-PAGE of LNFP-III–HSA (15 μg per lane) and HSA (12.5 μg per lane) was performed by the method of Laemmli (8). Sample buffer contained 2.5% 2-mercaptoethanol (Bio-Rad, Hercules, Calif.). Proteins separated by SDS-PAGE were electrophoretically transferred to a polyvinylidene fluoride membrane (Millipore, Bedford, Mass.) (22). The membrane was blocked in PBS-T0.3 for 1 h at room temperature. Individual membrane strips were subsequently incubated in sera diluted 1:800 in PBS-T0.3 or in undiluted culture supernatant of MAb E5 for 3 h at room temperature. After five washes with PBS-T0.3, membrane strips were incubated with alkaline phosphatase-conjugated goat anti-mouse IgM antibody (Boehringer Mannheim) diluted 1:1,000 in PBS-T0.3 for 1 h at room temperature. The strips were washed five times in PBS-T0.3, and signals were visualized by reaction with bromochloroindolylphosphate-nitroblue tetrazolium substrate according to the manufacturer's instructions (Bio-Rad). To visualize entire protein patterns, identical membrane strips, not exposed to antibodies, were stained with colloidal gold following the manufacturer's protocol (Diversified BioReagents, Newton Centre, Mass.).

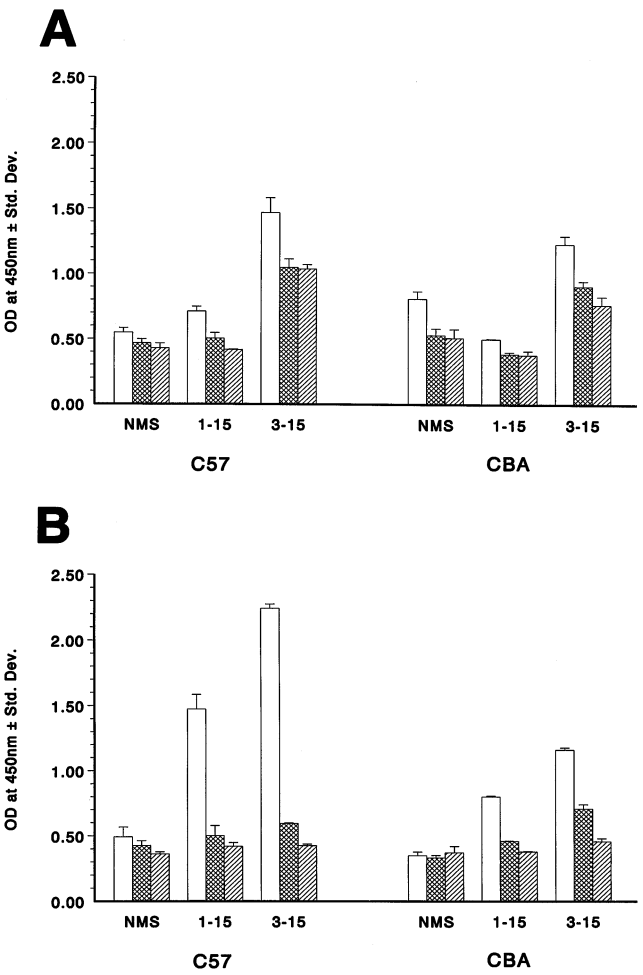

FIG. 1. Influence of serum dilution on CHO recognition in a membrane-enriched cercarial antigen preparation (DOC-CERC) using sera of mice vaccinated with irradiated cercariae. Sera were diluted 1:800 (A) or 1:1,600 (B). Levels of antibodies binding to DOC-CERC, either not treated (▭) or treated with 1 mM (▩) or 10 mM (▨) periodate, were measured by ELISAs. Sera were obtained from C57 and CBA mice vaccinated either once or three times with 15-kilorad-irradiated cercariae. Sera of naive mice (NMS) were used as negative control. Anti-IgG + IgM antibody served as probe. Std. Dev., standard deviation.

RESULTS

Vaccine sera recognize carbohydrate epitopes. To extend our earlier observation that VMS bind CHO epitopes on GST (18), we initially examined whether antibodies from vaccinated mice recognize CHO in DOC-CERC. Using ELISAs, we compared the binding of antibodies from C57 1-15, C57 3-15, CBA 1-15, and CBA 3-15 mice to native DOC-CERC or to DOC-CERC in which CHO had been denatured with periodate. When VMS were tested at a dilution of 1:800, the levels of antibodies recognizing DOC-CERC were not altered significantly following treatment with 1 or 10 mM periodate (Fig. 1A). However, at a serum dilution of 1:1,600, OD values of VMS binding to periodate-treated DOC-CERC were dramatically reduced to values similar to those of NMS controls (Fig.

1B). Experiments performed with a periodate concentration of 20 mM did not result in further reduction of antibody binding (data not shown). Thus, subsequent experiments using parasite antigen preparations were conducted with a serum dilution of 1:1,600 and a periodate concentration of 10 mM.

Vaccine sera detect carbohydrate epitopes in extracts of various schistosomal stages. To determine whether and to what extent antibodies in VMS recognize CHO epitopes in various stages of the parasite, we analyzed the antibody binding in C57 1-15, C57 3-15, C57 1-50, C57 3-50, CBA 1-15, CBA 3-15, CBA 1-50, and CBA 3-50 sera to saline-soluble or membrane-enriched larval, adult, and egg antigen preparations that were either treated with periodate or not treated. Because reduction of antibody binding to periodate-treated antigens compared with binding to nontreated antigens results directly from the loss of specifically recognized CHO epitopes, we deduce that glycosylated antigens were detected in CERC, SCHLAP, SWAP, SEA, DOC-CERC, DOC-SOM, and DOC-AD by most VMS tested (Table 1). For both mouse strains and virtually all antigen preparations, antibody titers to periodate-sensitive epitopes increased with multiple exposures to irradiated cercariae. Further, it was confirmed for all parasite stages examined that VMS from C57 mice contain higher antibody titers directed toward CHO than do VMS from CBA mice. Binding to CHO epitopes was reduced to lower levels when membrane-enriched antigen preparations of cercariae and adult worms were tested than when their saline-soluble counterparts were tested. Recognition of CHO epitopes appeared to be reduced most strongly in SEA and DOC-CERC.

LNFP-III is recognized by antibodies in vaccine sera. To identify particular CHO epitopes detected by VMS, we performed ELISAs testing for the presence of antibodies specific for LNFP-III. This oligosaccharide has been demonstrated previously in extracts of schistosomula and eggs and is recognized by the protective anti-egg MAb E5 (5). The presence of antibodies in VMS specific for its nonfucosylated homolog, LNT, was also examined. Both CHOs were covalently conjugated to HSA as the carrier protein. An anti-IgM antibody was used as the probe, because only this class has been reported to bind to LNFP-III (5). C57 1-15, C57 3-15 and CBA 3-15 sera contained significant levels of IgM antibodies to LNFP-III (Fig. 2A). In contrast, no LNFP-III-specific antibodies were detected in sera from C57 and CBA mice vaccinated once or three times with 50-kilorad-irradiated cercariae. The positive antibody controls, CMS, anti-egg MAb E5, and anti-SSEA1 MAb 3B11, bound strongly to LNFP-III, whereas the irrelevant anti-KLH MAb E3 did not bind. None of the VMS nor any of the MAbs recognized LNT (Fig. 2B). CMS, however, contained antibodies to this nonfucosylated oligosaccharide, with levels were lower than those specific for LNFP-III. Insignificant amounts of antibodies were measured, when the carrier protein HSA alone was tested (Fig. 2C). Thus, LNFP-III represents one of the CHO epitopes that are recognized by protective sera of mice vaccinated with irradiated cercariae.

By Western blot analysis, LNFP-III conjugated to HSA was recognized as a 72-kDa band by VMS and by the MAb E5 (Fig. 3). The intensity of binding to LNFP-III mirrored the results obtained by ELISA, sera from both C57 and CBA mice vaccinated with 15-kilorad-irradiated cercariae showed stronger binding than did sera from C57 and CBA mice vaccinated with 50-kilorad-irradiated cercariae, when tested at an identical dilution. VMS did not recognize the carrier molecule HSA at an approximate molecular mass of 67 kDa.

Both LNFP-III-specific MAbs are known to be of the IgM class (2, 5). To determine whether other antibody classes in VMS recognize this oligosaccharide, we used anti-IgG or anti-IgA antibodies as secondary antibody probes in ELISA. IgG antibodies present in VMS failed to recognize either LNFP-III or LNT at a significant level (Table 2). However, significant amounts of LNFP-III-specific IgA antibodies were measured in C57 1-15, C57 3-15, and C57 3-50 sera and to a lesser extent in CBA 3-15 and CBA 3-50 sera. Elevated levels of this antibody were also present in CMS of both C57 and CBA mice. The IgA response to LNT was weak or absent. Thus, VMS and CMS of both mouse strains appear to also contain antibodies of the IgA class that specifically recognize LNFP-III.

TABLE 1. Levels of antibodies in sera of mice vaccinated with irradiated cercariae recognizing CHOs in saline-soluble and membrane-enriched preparations of various stages of *S. mansoni*

Antigen[a]	Vaccination regimen[b]	C57 sera[c]		CBA sera[c]	
		Binding to native antigen	% reduced binding to treated antigen	Binding to native antigen	% reduced binding to treated antigen
CERC	1-15	1.50	44.7	0.88	18.1
	3-15	**2.53**	**62.6**	**1.23**	**29.8**
	1-50	0.73	29.8	0.68	5.9
	3-50	**1.67**	**67.4**	**1.36**	**43.5**
	–	0.49	0	0.60	0
SCHLAP	1-15	1.36	63.6	0.65	40.5
	3-15	**1.78**	**57.6**	**0.92**	**42.4**
	1-50	1.06	51.8	0.87	41.5
	3-50	**1.44**	**64.4**	**1.23**	**57.3**
	–	0.78	30.3	0.49	23.5
SWAP	1-15	1.34	37.5	0.81	19.7
	3-15	**2.24**	**51.7**	**1.22**	**38.4**
	1-50	0.83	21.5	0.95	13.2
	3-50	**1.30**	**46.5**	**0.87**	**16.5**
	–	0.68	4.3	0.81	0
SEA	1-15	1.47	81.6	0.48	53.0
	3-15	**2.12**	**86.6**	**0.77**	**68.5**
	1-50	0.72	66.9	0.49	28.0
	3-50	**1.28**	**80.0**	**0.80**	**54.1**
	–	0.41	32.2	0.23	0
DOC-CERC	1-15	1.47	71.5	0.80	52.6
	3-15	**2.24**	**81.0**	**1.16**	**60.5**
	1-50	0.94	62.3	0.85	50.1
	3-50	**1.62**	**77.6**	**1.53**	**69.9**
	–	0.43	15.6	0.35	0
DOC-SOM	1-15	1.53	40.4	1.05	28.3
	3-15	**2.27**	**54.9**	**1.73**	**55.1**
	1-50	1.18	40.8	1.01	20.5
	3-50	**1.50**	**54.8**	**1.61**	**45.3**
	–	0.63	2.7	0.65	0
DOC-AD	1-15	1.30	64.7	0.62	43.3
	3-15	**2.08**	**76.8**	**1.14**	**66.6**
	1-50	0.70	53.6	0.45	20.3
	3-50	**1.09**	**69.7**	**0.64**	**37.0**
	–	0.46	9.7	0.39	7.1

[a] Saline-soluble antigen preparations of cercariae (CERC), schistosomula (SCHLAP), adult worms (SWAP), eggs (SEA); membrane-enriched antigen preparations of cercariae (DOC-CERC), schistosomula (DOC-SOM), and adult worms (DOC-AD).

[b] Sera were obtained from mice vaccinated once or three times with 15- or 50-kilorad-irradiated cercariae or from naive mice (–) and diluted 1:1,600.

[c] Levels of anti-CHO antibodies, calculated from OD values derived from antibody binding to antigens, treated with 10 mM periodate, divided by OD values derived from antibody binding to nontreated antigens and multiplied by 100.

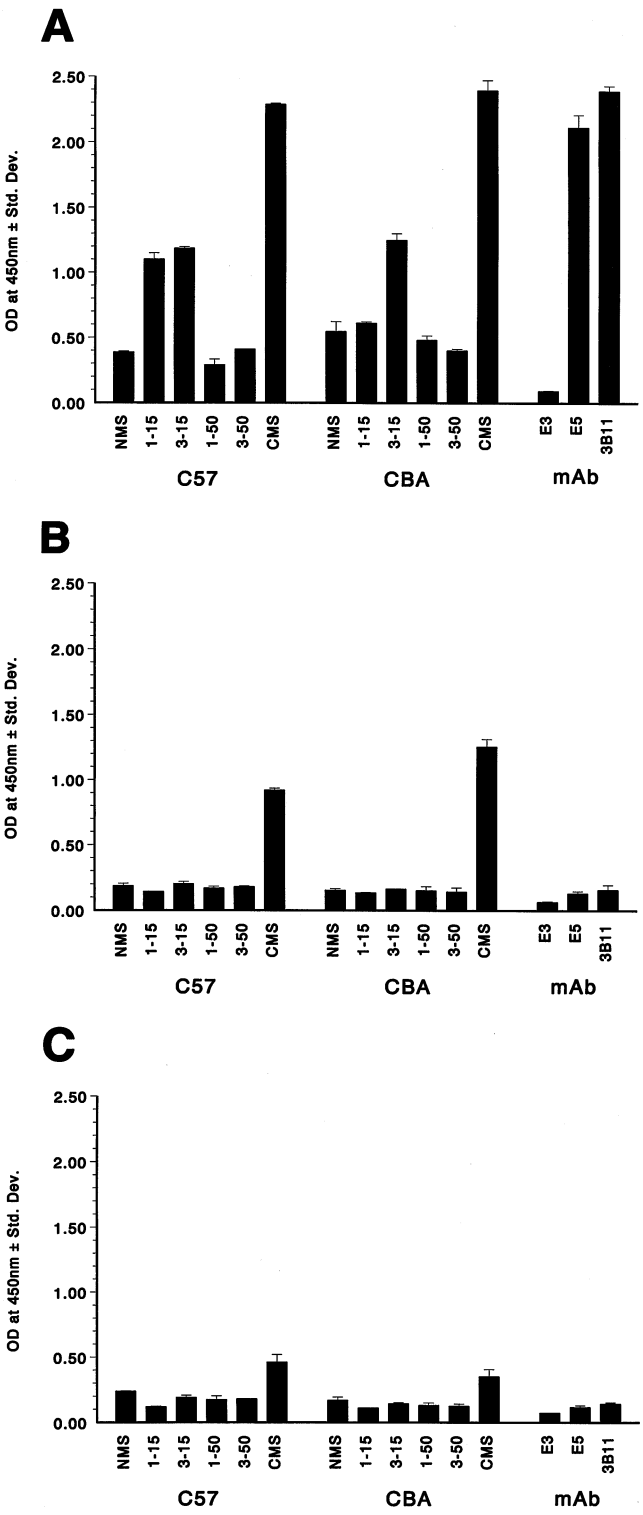

FIG. 2. Recognition of LNFP-III by sera of mice vaccinated with irradiated cercariae. Levels of antibodies binding to LNFP-III (A), to its nonfucosylated homolog LNT (B), or to the carrier molecule HSA (C) were measured by ELISAs. Sera of C57 and CBA mice vaccinated either once or three times with 15- or 50-kilorad-irradiated cercariae were diluted 1:800. Sera of naive mice (NMS) and anti-KLH MAb (E3) were used as negative controls, and sera of chronically infected mice (CMS) and MAbs E5 and 3B11 were used as positive controls. Anti-IgM antibodies served as the probe. Std. Dev., standard deviation.

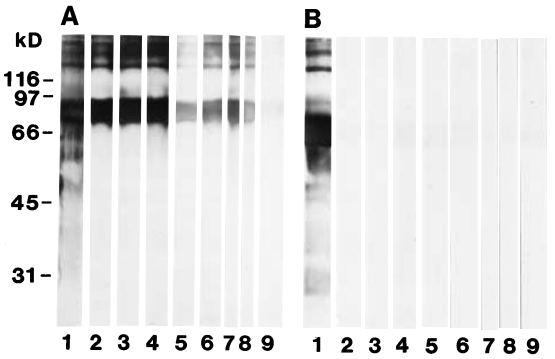

FIG. 3. Recognition of LNFP-III by sera of mice vaccinated with irradiated cercariae. Western blot analysis of LNFP-III conjugated to HSA (A) and of the carrier molecule HSA (B) probed with sera of C57 1-15 (lanes 3), C57 3-15 (lanes 4), C57 1-50 (lanes 5), C57 3-50 (lanes 6), CBA 3-15 (lanes 7), and CBA 3-50 (lanes 8) mice or with MAb E5 (lanes 2) or incubated with colloidal gold (lanes 1). Sera of naive mice served as negative control (lanes 9). SDS-PAGE was run under reducing conditions.

Vaccine sera do not recognize carbohydrate epitopes on KLH. KLH contains cross-reactive CHO epitopes that are shared by schistosomula and eggs (1). We performed ELISAs probing native and periodate-treated KLH with various VMS. None of the VMS contained antibodies specific for epitopes on KLH (Fig. 4). Antibodies in CMS bound weakly to epitopes of KLH. These epitopes seemed to be peptides, because no reduction in binding was observed when periodate-treated KLH was used. The positive control, MAb E3, strongly bound to native KLH, but not to periodate-treated KLH, confirming its specificity for a CHO epitope.

DISCUSSION

The present study demonstrates that sera from mice vaccinated with irradiated cercariae contain high levels of antibodies specific for CHO epitopes in saline-soluble or membrane-enriched antigen preparations from various stages of *S. mansoni*. Failure of earlier studies to detect CHO-specific antibodies in VMS may have resulted from the high serum concentration (1:10) used (15, 16). Indeed, in our study, the anti-CHO response correlated negatively with increasing serum concentrations. Thus, antipeptide antibodies may be present at lower titers than anti-CHO antibodies but perhaps may sterically block the binding of CHO-specific antibodies, if antipeptide antibodies saturate their epitopes.

The genetic background of vaccinated mice seems to influence the humoral response directed toward glycosylated epitopes, because C57 mice produced significantly more anti-CHO antibodies than did CBA mice. The highest levels of anti-CHO antibodies appeared to be present in C57-15 mice, the group that generates the highest level of resistance to challenge (17, 18).

One of the CHO epitopes recognized by protective VMS was identified as the oligosaccharide LNFP-III. Analogous to the antibody response observed to overall glycosylated epitopes in crude antigen preparations, the humoral recognition of LNFP-III varied somewhat for the two mouse strains. Whereas vaccinated C57 mice produced LNFP-III-specific antibodies after the first exposure to irradiated cercariae, CBA mice did so only after two additional exposures. The irradiation dose of the immunizing cercariae also affected the humoral response to this particular oligosaccharide epitope. LNFP-III-specific antibodies appeared to be restricted to sera from mice vaccinated

TABLE 2. Classes of antibodies binding to LNFP-III

Mouse strain	Vaccination regimen[a]	Level of antibody binding[b]					
		LNFP-III–HSA		LNT-HSA		HSA	
		IgA	IgG	IgA	IgG	IgA	IgG
C57	1-15	0.35 ± 0.04	0.05 ± 0.02	0.12 ± 0.02	0.03 ± 0.01	0.06 ± 0.05	0.02 ± 0
	3-15	0.46 ± 0.03	0.11 ± 0	0.12 ± 0.02	0.06 ± 0	0.14 ± 0.08	0.05 ± 0.01
	1-50	0.24 ± 0.06	0.04 ± 0.01	0.14 ± 0.01	0.03 ± 0	0.17 ± 0.04	0.03 ± 0
	3-50	0.40 ± 0.02	0.08 ± 0.01	0.19 ± 0.06	0.04 ± 0.02	0.23 ± 0.06	0.06 ± 0
	Infected	0.87 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.04 ± 0	0.19 ± 0.04	0.03 ± 0
	Naive	0.20 ± 0.01	0.09 ± 0	0.12 ± 0	0.07 ± 0	0.16 ± 0.04	0.08 ± 0.01
CBA	1-15	0.10 ± 0.05	0.05 ± 0.01	0.13 ± 0.05	0.06 ± 0	0.00 ± 0.05	0.02 ± 0
	3-15	0.21 ± 0.06	0.06 ± 0	0.18 ± 0.04	0.04 ± 0	0.02 ± 0.01	0.01 ± 0
	1-50	0.11 ± 0.03	0.09 ± 0.01	0.16 ± 0.01	0.11 ± 0	0.04 ± 0.01	0.06 ± 0.01
	3-50	0.21 ± 0	0.06 ± 0	0.14 ± 0.02	0.05 ± 0	0.00 ± 0.06	0.03 ± 0
	Infected	0.53 ± 0	0.22 ± 0.01	0.09 ± 0.01	0.16 ± 0	0.05 ± 0.04	0.10 ± 0
	Naive	0.24 ± 0.07	0.06 ± 0.01	0.16 ± 0.03	0.08 ± 0.02	0.06 ± 0.03	0.03 ± 0.01

[a] Sera were obtained from mice vaccinated once or three times with 15- or 50-kilorad-irradiated cercariae, from infected mice, or from naive mice and diluted 1:800.
[b] Levels (measured as OD ± standard deviation) of IgA and IgG antibodies binding to LNFP-III, to its nonfucosylated homolog LNT, or to the carrier molecule HSA.

with 15-kilorad-irradiated cercariae. Sera from mice vaccinated with less-protective 50-kilorad-irradiated cercariae contained no or barely detectable amounts of IgM antibodies recognizing LNFP-III. Parasites irradiated with 50 kilorads may not survive long enough to stimulate a measurable response. Additionally, expression of this epitope on the surface of the parasite is developmentally regulated and initiated only after transformation to the schistosomulum stage (7); thus, irradiation may have an effect on its synthesis. Taken together, LNFP-III is one of the carbohydrate targets of sera from mice repeatedly vaccinated with 15-kilorad-irradiated cercariae (3-15 VMS), which were previously shown to be protective in passive transfer experiments.

The glycoprotein KLH contains CHO epitopes that cross-react with surface membrane antigens of cercariae and schistosomula and with saline-soluble egg antigens (1). Sera of both patients and experimentally infected animals contain antibodies to cross-reactive CHOs on KLH (1). Nevertheless, we observed no binding to KLH by antibodies in VMS, suggesting that the anti-CHO response of these sera is fairly restricted.

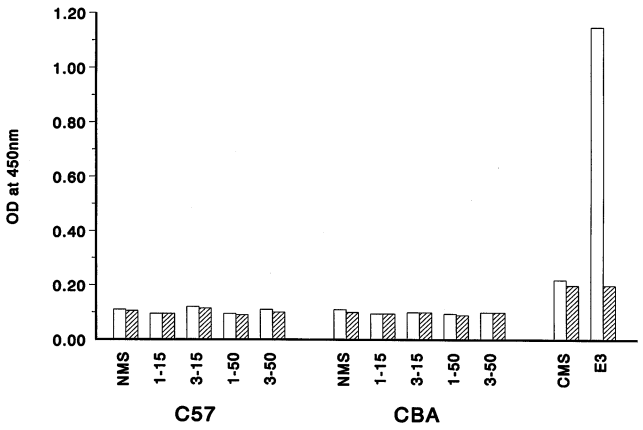

FIG. 4. No recognition of KLH by sera of mice vaccinated with irradiated cercariae. Levels of antibodies binding to epitopes on KLH, either not treated (▭) or treated with 10 mM periodate (▨) were measured by ELISAs. Sera were obtained from C57 and CBA mice vaccinated either once or three times with 15- or 50-kilorad-irradiated cercariae. Sera of naive mice (NMS) were used as negative control, and sera of chronically infected mice (CMS) and anti-KLH MAb (E3) were used as positive controls. Anti-IgG + IgM antibody served as probe.

Sera from C57 3-15 CBA 3-15 mice confer high levels of resistance to challenge infection, when transferred to naive mice (18). The LNFP-III-specific MAb E5 is similarly protective upon passive transfer (6) and mediates effector cells to kill schistosomula in vitro, as does another LNFP-III-specific MAb, the anti-SSEA1 MAb (3B11) (5). All MAbs hitherto known to recognize LNFP-III (E5, anti-SSEA1, and 504B1) are of the IgM class (5, 7). Similarly, mice of a variety of different strains are able to generate IgM but not IgG antibodies specific for LNFP-III (23). In agreement with these previous studies, anti-LNFP-III antibodies present in VMS were predominantly of the IgM class. No IgG antibodies bound to this oligosaccharide, suggesting a further explanation for the failure of earlier studies to detect anti-CHO antibodies in VMS (15, 16). Interestingly, in the present study, significant levels of IgA antibodies specifically recognized LNFP-III in VMS and CMS. This IgA response was more pronounced in C57 mice. Thus, antibodies of not only the IgM class but also of the IgA class bind to LNFP-III.

VMS recognized more CHO epitopes in membrane-enriched extracts than in saline-soluble preparations from the same stages, reflecting the general observation that glycosylated proteins are predominantly surface exposed (15). LNFP-III is also expressed on the parasite's surface, as demonstrated by immunolocalization studies (5, 7). VMS detected most CHO epitopes in SEA, which is consistent with previous studies demonstrating cross-reactive epitopes on schistosomula and eggs. LNFP-III appears to be one of these cross-reactive epitopes, because both LNFP-III-specific MAbs, E5 and 504B1, were derived from mice immunized with egg antigens (5, 7). We demonstrated recently that LNFP-III is present in higher concentrations on GST isolated from eggs than on GST derived from adult worms (27). Indeed, VMS contains antibodies specific for CHO epitopes on GST (18).

The presence of LNFP-III-specific antibodies in VMS suggests that migrating attenuated larvae stimulate this response, because LNFP-III expression on the surface is initiated shortly after transformation to the schistosomulum stage (5, 25). LNFP-III expression by the parasite may have several impli-

cations for the host-parasite relationship. The LNFP-III molecule contains the trisaccharide Lewis^x which is expressed in its sialylated form on blood cells of the host. Sialylated Lewis^x serves as strong ligand for several selectins, such as P-selectin CD62 (PADGEM and GMP-140) found on endothelial cells and platelets, and mediates adhesion of granulocytes and monocytes during blood clotting (13, 14, 21). LNFP-III and LNFP-I are also ligands for the lung endothelial cell adhesion molecule 1. In general, selectin-ligand interactions serve to direct specific cell types to their target sites. Therefore, it is tempting to propose that the presence of LNFP-III on the parasite may be relevant for its migration to the lungs and eventually to its final destination in the portal system. In addition to antibody recognition, we demonstrated recently that schistosomal LNFP-III also interacts with B220$^+$ CD4$^-$ CD8$^-$ cells of infected and naive mice (24). This oligosaccharide appears to induce the production of interleukin 10 and prostaglandin E_2 that down-regulate T_H1 cells and stimulate expansion of T_H2 cells. In conclusion, the conceivable interactions between schistosomal LNFP-III with host selectins or immunoglobulins seem to be remarkably diverse. The protective effect of a candidate vaccine antigen, therefore, may be influenced by the presence of LNFP-III and other CHOs serving as ligands.

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